

***Archophileurus spinosus* Dechambre, 2006 (Coleoptera: Scarabaeidae: Dynastinae), a new exotic scarab possibly acclimatized in Italy, with a compilation of exotic Scarabaeidae found in Europe**

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Abstract

Archophileurus spinosus Dechambre, 2006, a South American Scarabaeidae (Coleoptera: Scarabaeidae: Dynastinae: Phileurini) is recorded for the first time in Europe (Apulia, Italy). This species, which may have colonized the Italian peninsula in recent past and that could still be in the acclimatization phase, represents the first record of tribe Phileurini in Europe and the second case worldwide of interception of this genus outside its area of origin. After a brief presentation of the site and collecting conditions of the specimens, we discuss the possible threats for local biodiversity and agriculture that the establishment of *A. spinosus* could cause.

Key words: Alien, Coleoptera, Europe, exotic, globalization, IAS, Scarabaeoidea, threat

Introduction

International trade is the primary cause of introduction and spreading of invasive alien species globally. Invasive alien species often constitute a serious threat for ecosystem balance, biodiversity conservation and cause relevant economic damage to human activities (Kenis & Branco 2010; Pyšek & Richardson 2010; Cameron *et al.* 2016); consequently, allochthonous species monitoring and management are among the most challenging tasks to accomplish nowadays. Early detection plays a central role in the control of invasive alien species, especially those that escape the surveillance implemented at the most common points of entry, such as ports and airports (Liebholt & Tobin 2008; Hulme 2009; Poland & Rassati 2019). Exotic species spreading is directly linked to ecosystem degradation in many parts of the world, including the Mediterranean-climate zones: the Mediterranean basin in particular is one of the biodiversity hotspots most at risk due to its pivotal role in world trade (Katsanevakis *et al.* 2014).

Scarabaeidae are a large and extremely diverse family of Coleoptera capable to successfully adapt to the majority of ecological niches. Thanks to this diversity in adaptations, biology and size, representatives of this group have often been introduced actively or passively by man all over the world (*e.g.* Barbero & Loípez-Guerrero 1992; Dymock 1993; Jameson *et al.* 2009). Some of these species are now common and widely recognized pests (*e.g.* Abdallah *et al.* 2013; Allsopp *et al.*, 2018). This dispersal and establishing process seems to be favored by the progressive global warming, especially in temperate climates. In Europe, more than fifteen exotic scarab species have been recorded so far (Table 1).

In the present paper we report the first record of *Archophileurus spinosus* Dechambre, 2006 (Scarabaeidae: Dynastinae: Phileurini) in Europe, based on four specimens collected between 2017 and 2019 in southern Italy. Furthermore, we discuss the possible introduction pathway of this scarab, trying to define the potential risks that a stable population of this species could cause to the environment and the agricultural activities.

TABLE 1. List of exotic Scarabaeidae intercepted or established in Europe

Species	Subfamily	Origin	Record	References
<i>Acrossidius tasmaniae</i> (Hope, 1847)	Aphodiinae	Oceania	Italy	Mazza <i>et al.</i> 2014
<i>Aphodius gracilis</i> Boheman, 1857	Aphodiinae	Africa	Portugal-Azores	Denux & Zagatti 2010
<i>Ataenius brevicollis</i> (Wollaston, 1854)	Aphodiinae	Azores	Portugal-Azores	Bezděk 2016
<i>Ataenius gracilis</i> (Melsheimer, 1844)	Aphodiinae	South America	Portugal-Azores	Bezděk 2016
<i>Ataenius picinus</i> Harold, 1867 (species identity requires confirmation, G.M. Carpaneto, personal communication)	Aphodiinae	South America	France, Italy	Inghilesi <i>et al.</i> 2011; Lemaire & Raffaldi 2014; Leo <i>et al.</i> 2015; Bezděk 2016
<i>Ataenius simplicipes</i> Mulsant & Rey, 1870	Aphodiinae	South America	France	Bezděk 2016
<i>Ataenius spretulus</i> (Haldeman, 1848)	Aphodiinae	North America	Portugal	Branco 2011; Leo <i>et al.</i> 2015; Bezděk 2016
<i>Parataenius simulator</i> (Harold, 1868)	Aphodiinae	South America	Portugal	Inghilesi <i>et al.</i> 2011; Bezděk 2016
<i>Saprosites mendax</i> (Blackburn, 1892)	Aphodiinae	Australasia	Great Britain	Angus <i>et al.</i> 2003; Denux & Zagatti 2010; Branco 2011; Inghilesi <i>et al.</i> 2011; Bezděk 2016
<i>Saprosites natalensis</i> (Péringuey, 1901)	Aphodiinae	Africa	Great Britain	Angus <i>et al.</i> 2003; Branco 2011; Bezděk 2016
<i>Saprosites peregrinus</i> Redtenbacher, 1858	Aphodiinae	Central-South America	Austria	Branco 2011; Bezděk 2016
<i>Tesarius caelatus</i> (LeConte, 1857)	Aphodiinae	North America	Great Britain	Johnson 1975; Rakovič <i>et al.</i> 2016
<i>Tesarius mcclayi</i> (Cartwright, 1955)	Aphodiinae	North America	Great Britain	Rakovič <i>et al.</i> 2016
<i>Chiron cylindrus</i> Fabricius, 1798	Chironinae	Asia	France, Italy	Král & Bezděk 2016
<i>Augosoma centaurus</i> (Fabricius, 1775)	Dynastinae	Africa	Italy	Ratti 2007b
<i>Pentodon algerinus dispar</i> Baudi, 1870	Dynastinae	Middle East	Germany	Baufeld & Schrader 2017
<i>Pentodon variolopunctatus deserti</i> (Heyden, 1900)	Dynastinae	North Africa and Middle East	Bosnia and Herzegovina	Krell & Bezděk 2016
<i>Temnorhynchus baal</i> Reiche & Sauley, 1856	Dynastinae	South-East Europe	Sweden	Forshage & Krell 2016
<i>Tomarus villosus</i> (Burmeister, 1847)	Dynastinae	South America	Sweden	Forshage & Krell 2016
<i>Liogenys excisa</i> (Reitter, 1918)	Melolonthinae	South America	Italy	Sanmartín & Martín-Piera 2003; Ratti 2007a
<i>Serica intermixta</i> Blatchley, 1910	Melolonthinae	North America	Germany	Ahrens & Kless 2001; Ahrens & Bezděk 2016
<i>Euonicellus intermedius</i> (Reiche, 1850)	Scarabaeinae	Africa	Italy	Ratti 2007a
<i>Anomala varicolor</i> (Gyllenhal, 1817)	Rutelinae	Southeast Asia	Italy	Paulian & Baraud 1982
<i>Popillia japonica</i> Newman, 1841	Rutelinae	Asia	Italy, Switzerland, Germany, Portugal-Azores	Urban, 2018; Eppo 2019; Urban <i>et al.</i> 2019

Materials and methods

The four specimens (see Material examined for details) of *A. spinosus* studied come from Serrano, a village in the province of Lecce (Apulia, Italy). The insects were apparently attracted by the lights used to illuminate monuments and sports facilities. The female was kept alive in captivity to assess whether it was fertile and capable to lay eggs. At the time of drafting this article and before submission, the female was able to lay two eggs, none of which has hatched yet. Species identification is based on the single male available (Fig. 1) using the keys provided by Endrődi (1977) and Di Iorio *et al.* (2017). Molecular analyses were conducted on the two last specimens collected. The DNA was extracted from each individual separately using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. A region of mitochondrial DNA, corresponding to a fragment of the COI gene, was amplified using the universal primers LCO-1490/HCO-2198 (Folmer *et al.* 1994). Amplifications were performed in 20 µl reactions (1 × polymerase chain reaction (PCR) Go Taq Flexi buffer—Promega, 2.5 mM MgCl₂, 0.1 mM dNTPs, 0.5 µM for each primer, 0.5 U of Taq polymerase—Promega, 2 µl DNA template). Thermal cycling conditions were 5 min at 96°C followed by four cycles of 96°C for 1 min, 48°C for 1 min, and 72°C for 1 min, and other 35 cycles of 96°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min. PCR products were purified using Exonuclease and Antarctic Phosphatase (GE Healthcare) and sequenced at the BMR Genomics Service (Padova, Italy). Sequences were edited and aligned using MEGA X (Kumar 2018) and subsequently, translated with Transeq (EMBOSS: <http://www.ebi.ac.uk/Tools/emboss/transeq/index.html>) to exclude the presence of stop codons in the coding region. A GenBank BLAST analysis of the sequences obtained was run through the NCBI website (<http://www.ncbi.nlm.nih.gov>) and the integrated bioinformatics platform Barcode of Life Data (BOLD) System database (<http://www.barcodinglife.org>) was used to assess the identity of the sequences.

Archophileurus spinosus Dechambre, 2006

Material examined. Italy, Apulia: Serrano, 02. viii. 2017, 40°11'06.5"N 18°21'02.3"E, F. Tomasi lgt., dead female; Serrano, 03. vii. 2019, 40°10'58.5"N 18°21'15.5"E, F. Tomasi lgt., dead male; Serrano, 05. vii. 2019, 40°10'58.7"N 18°21'08.5"E, F. Tomasi lgt., live female; Serrano, 02. viii. 2019, 40°11'02.2"N 18°21'04.4"E, F. Tomasi lgt., dead female.

All specimens are deposited in the personal collection of Filippo Tomasi (Serrano, Italy).

Systematics, biology and distribution

Archophileurus Kolbe, 1910 includes more than 30 described species (Di Iorio *et al.* 2017) and it is widely distributed throughout the entire New World, from South United States (Texas) to the northern part of Argentina and Chile (Moron 1990; Di Iorio *et al.* 2017). The genus is morphologically homogenous, and species identification is possible with certainty almost exclusively on the basis of the examination of male genitalia. Females are particularly difficult to identify and, in many cases, undescribed. Very little is known about the biology of both adult and larvae of Phileurini, especially for *Archophileurus*. The larvae of this genus generally develop in rotten and decaying wood (Moron 2009) as well as termite nests (Vanin *et al.* 1983), while adults are predators of other beetle larvae, such as Tenebrionidae and Passalidae (Moron 2009). *Archophileurus* larvae, as member of the “white grubs” guild (“gusanos blancos”), are also reported as possible cause of damage on crops, wheat and *Citrus* sp. especially (Ritcher 1966; Alzugaray *et al.* 1999; Di Iorio 2004; Massaro 2010; Mondaca 2011); however, the true harmfulness of this genus is still to be clarified.

Archophileurus spinosus was originally described from Paraguay (Dechambre 2006) and subsequently recorded in Northern, Central and Eastern Argentina (Di Iorio *et al.* 2017); its biology and the larval morphology remain unknown. The only existing record of the accidental introduction of *Archophileurus* sp. outside its natural range refers to a unique case happened in the USA (Meissner *et al.* 2009). *Archophileurus* species, like many other Phileurini, seem to prefer tropical forests (Di Iorio *et al.* 2017) even if the genus successfully colonized also harsh and xeric environments (Moron 1990).

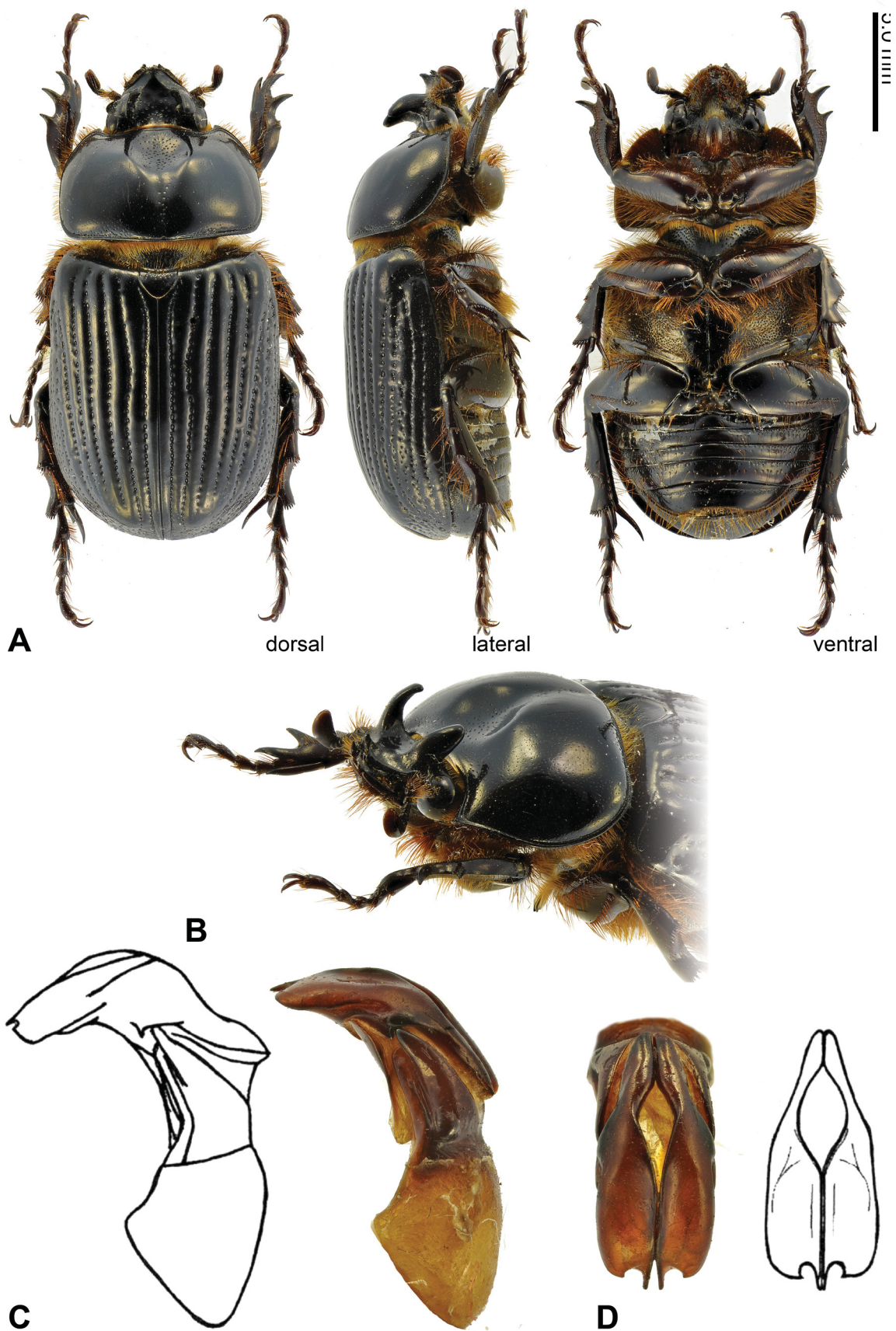


FIGURE 1. Male of *Archophileurus spinosus* Dechambre, 2006 from Serrano (Italy). A, dorsal, lateral and ventral views; B, dorso-lateral view of the head; C, aedeagus in lateral view and original drawing from Di Iorio *et al.* (2017); D, parameres in dorsal view and original drawing from Di Iorio *et al.* (2017).

Diagnosis

Adult male: *A. spinosus* closely resembles in general aspect and body size *Archophileurus chaconus* (Kolbe, 1910) and *Archophileurus vervex* (Burmeister, 1847) and the three species can be separated only on the base of parameres shape (see Di Iorio *et al.* 2017). Both females and larvae of the entire genus are not enough characterized to provide any discrimination character to separate one species from another; for this reason, DNA barcoding is certainly a tool of great help in the rapid identification of samples. A fragment of the mitochondrial gene COI (the barcode region) 631 bp long was obtained and sequences of the two individuals analyzed were identical; we here provide the COI sequence of *A. spinosus*: TGG AACATCACTAAGACTCTTAATCCGGGCTGAACTAGGAAACCCCGGATCCCTCATTGGA-GATGATCAAATTTATAACGTAATTGTTACAGCCCACGCTTTTATTATAATTTCTTCATAGTTATACCAAT-TATAATTGGGGGATTTCGGAACTGACTAGTACCCCTAATACTAGGAGCCCCTGATATAGCATTCCAC-GAATAAATAATATAAGATTTTGACTTTTACCCCCATCCCTAACGCTTCTTTTAGCTAGAAGAGTAG-TAGAAAATGGTGCAGGAACAGGCTGAACTGTCTATCCACCCTTATCAGCCAACATTGCTCATAGAG-GAGCCTCTGTAGACTTAGCAATTTTCAGATTACACCTCGCAGGAATCTCATCAATCTTGGGAGCTGTA-AATTTTCATTACCACAGTAATCAACATACGATCAACAGGAATAACTTTTGACCGTATACCCCTATTCGCTGATC-GGTAGTATTAATACTGCAATCTTATTACTTCTATCACTACCAGTTCTTGCAGGAGCAATTACAATACTCCTA-ACAGATCGAAATATTAACACCTCTTTTTTTGATCCCGCAGGAGGTGGGGACCCAATCCTCTACCAA-CATTTATTTTGATTTTTTGTC.

A comparison with BoldSystem and NCBI databases showed a similarity of 86.6% and 83.7% respectively with *Allidiostoma* sp. (Allidiostomatinae) and surprisingly only an 83.96% similarity with *Archophileurus simplex* (Bates, 1888) (Costa Rica).

Discussion

Italy is a country at high risk of introduction of exotic species due to the important commercial and tourist traffic and to its particularly favorable climatic and environmental conditions (Jucker & Lupi 2011). For these reasons, the degree of attention towards exotic species remains particularly high. Every year new exotic beetles are intercepted in the main arrival points; however, these likely constitute only a limited fraction of the actual arrivals. It is interesting to note that most of the exotic species recently found in Italy have been captured in the natural environment already, and they probably have stable populations (Clark *et al.* 2014; Zappi 2014; Salvato & Uliana 2016; Toma *et al.* 2017; Guariento *et al.* 2019; Mola & Yoshida 2019; Forbicioni 2019; Ruzzier & Colla 2019). The presence of *Archophileuru spinosus* in Apulia, although totally unexpected, is perfectly in line with the aforementioned records: beetles that usually escape the main control procedures or that are not targeted by the most-used monitoring systems. This taxon despite having only recently been described and being known in a limited number of specimens, is apparently widely distributed in Argentina, Paraguay and potentially could occur in Uruguay and South Brazil (Di Iorio *et al.* 2017). *Archophileurus spinosus* seems to be rarer than the closely related *A. chaconus* and *A. vervex*, however its wide distribution has probably made it more predisposed to be transported outside its natural range. The scant information available about *A. spinosus* biology combined with the scarce data deriving from the place of its discovery therefore make it difficult to reconstruct the pathway of introduction of this species into the Italian territory, especially considering that Serrano is a village lacking any important industrial areas and being located far from international ports and airports. As a consequence, we can only hypothesize that this species may have been introduced at the larval stage in association with some commodities such as ornamental plants and/or soil for gardening, as it happened with many other Scarabaeoidea (Forshage & Krell 2016; Allsopp *et al.* 2018). The escape of some specimens from amateur breeding has to be discarded, as the species does not belong to those genera usually bred and traded; furthermore, almost nobody has ever tried rearing *Archophileurus* (Paschoal C. Grossi, personal communication). The limited data available on *Archophileurus* biology, both for adults and larvae, makes it difficult to estimate the possible impacts that this species may have on the local ecosystem and agriculture. Similarly to those of *A. vervex* or *A. chaconus*, the larvae of *A. spinosus* may cause damages to the roots of cultivated plants in the new environment of southern Italy. Furthermore, if the adults of this species were predators of larvae of other beetles, they could have an impact on the local fauna. In the area, *Phyllognathus excavatus* (Forster, 1771) and *Oryctes nasicornis* (Linnaeus, 1758) (Scarabaeidae: Dynastinae) are common (F. Tomasi, personal observation) and the larvae of the latter develop in numbers in the mulching of the olive groves. This type of environment could constitute, in

the absence of large hollow trees, the ideal reproduction site for *A. spinosus*; there the larvae would have a sufficient and stable substrate to develop while the adults could prey on the larvae of the other scarabs. Furthermore, given the predisposition of this genus to colonizing decaying trees and their cavities, a possible range expansion of this species on the Italian territory could put at great risk different groups of saproxylic beetles, some of which are already in conditions of serious threat (Audisio *et al.* 2014).

At the moment it is of primary interest to define whether a stable population of *Archophileurus spinosus* species exists and if this species is present in other localities. We believe it is necessary to investigate the origin of these specimens, confirm the presence of a stable population and eventually develop a prompt monitoring strategy so as to identify the possible effects that this taxon may have on the ecosystem and in agricultural systems. It is in fact in these early stages of invasion that containment and eradication plans are more likely to succeed. We also hope that DNA barcoding will be of help to research groups and phytosanitary institutes in identifying this species from the larval stages, so as to carry out control actions well before the appearance of adults.

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